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May 14, 1950

Dear Joshua:

My long silence has represented a period of failure and doubt out of which I now seem to be emerging, just 18 days before the end of my year's leave. The Streptomycin-Azide selection for complementaries failed totally, even though practically all MTL prototrophs are doubly sensitive. In 42 complementary pour-plates in two experiments I found only 4 colonies, and these were all mutants from one of the parental strains. The explanation is apparently that, while streptomycin does not seem to be linked to TL, neither is it linked to M, because in one Strep-Azide cross I made, both Methionine and T-L loci behaved as though closer to Azide than to streptomycin. Furthermore, the only 4 complementaries I have tested for Streptomycin and azide resistance were sensitive to streptomycin; 3 were resistant to azide, about as anticipated. In general, the linkage data from the strep-azide cross don't make sense with respect to any loci under the hypothesis of linear linkage, but the distortions introduced by selection might conceivably account for this.

A second experiment testing P B_1 prototrophs confirms my earlier finding of nearly 100% crossing over between proline and T+. Figure that one out*! My most successful single experiment with MTL prototrophs yields a map like this (distances in the M-TL interval only relative):(based on 102 recombinants):

B_1	M	Ia	P	Lac		V_1	Az	T-L
5	7	17	7		49	27	11	

Aside from reasonable fluctuations with respect to some of the distances as determined from other experiments, the only non-linear observation in this experiment was the low figure of 48% crossing over between proline and V_1 , compared with 49% between Lac and V_1.

I seem to have two loci for Gal-plus, one giving only a faint color reaction, unreliable, located between P and Lac, and another giving a full color reaction and behaving in its linkage like Maltose, but not linked to maltose. My T_6 data are, so far, unreliable.

My big trouble at present is the rarety of recovery of complementaries, so that I have as yet no conclusive statistical or linkage evidence bearing on the question of 4-strand crossing over. This week I hope to try out two modifications that may solve the problem. First, I am going to try enriching the cross plates not only with P and B_1, but with infinitesimal amounts of MTL so that the complementary cells can undergo more multiplication within the partial-prototroph colony. Of course that will increase the danger of contamination with unrelated recombinants, but if most of the growth on complementary plates are sister segregants they should still provide the necessary statistical and linkage proof of the hypothesis. Second, I'm going to try to select complementaries with Azide and Iodoacetate instead of with Azide and Streptomycin.

* I still think I must somehow be selecting for T+.

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The search for quadruples was badly planned and has now been abandoned. Really, triples are quite adequate for the purpose, and if the iodo-acetate and azide method is successful, double amino acid requriements are enough. We got as far as triples and then found that the stocks didn't yield useful numbers of prototrophs in any combination. Now we have a prolineless-something else that Tom Nelson gave us that crosses very well with cystine-phenylalanine (Y-24), and if time permits I want to see if I can get complementariesfrom that cross, and, if so, if the streptomycin locus behaves as in the original cross.

A further advantage of the drug method of selecting complementaries, which I hadn't thought of originally, is the fact that the types recovered are not limited in any respect except double resistance, and can fairly be compared with recombinants selected in the ordinary way with inhibitors. Using growth factors alone, on the other hand, the complementaries must not only have the two selected factors, but they cannot be prototrophic for the factors selected in the original cross; thus, with the method I've been using, $B_1^+ P^+ T^+ L^+ M^+$ could never occur among the complementaries, though it would probably be fairly common (18% of 44) among $B_1^+ P^+$ recombinants in general. Now I'm wrong again; of course the complementaries could still not be prototrophic for the selected factors or they would constitute a major part of the original colony, but the difference is that with factors so closely linked to the selected loci as Ia and Az, the MTL prototrophs would constitute less than 1% of a control series of $I^r A^r$ recombinants.

With my time running so short now, I'll probably not write you again until after I've returned to medical school, when I'll presumably be working on a manuscript.

Give my regards to Ester.

Sincerely yours,

Gordon